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Origin and Relationships of the Sino-Himalayan *Impatiens* (Balsaminaceae) Based on Molecular Phylogenetic Analysis, Chromosome Numbers and Gross Morphology

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We used the combined sequence of *rbcL* and the spacer between *trnL* and *trnF* to generate the first molecular phylogeny of *Impatiens* (Balsaminaceae) based on twenty five species of *Impatiens* and using *Tropaeolum major* L. (Tropaeolaceae) as the outgroup. The phylogenetic tree drawn by this analysis revealed the presence of two large monophyletic clades: one consisting only of Himalayan species and the other of species distributed in various areas of the world. The phylogenetic tree is strongly correlated with chromosome numbers reported for the species examined: the Himalayan clade has $x = 7$ or 9; the other clade has $x = 8$ or 10. With regard to character evolution, the tree supported evolutionary trends in basic chromosome numbers and inflorescence morphology. Morphological diversification of the lower sepal, which is a significant characters used in the classification at the species level, does not clearly correlate with the tree.

Key words: Balsaminaceae, *Impatiens*, inflorescence, molecular systematics, Sino-Himalaya.

Impatiens L. (Balsaminaceae) consists of from 450 to 850 species distributed in tropical Africa and India and in the northern hemisphere with concentrations of species in tropical Africa, southeast Asia, and the Sino-Himalayan region (Grey-Wilson 1980, Cronquist 1981). The distinctive zygomorphic entomophilous flowers, with the lower sepal developing a nectariferous spur, show coevolutionary relationships with their pollinators (Grey-Wilson 1980). *Impatiens* is one of the largest genera in the world and exhibits tremendous morphological and cytogenetical diversity and species level complexity.

Systematic difficulties also exist at the infrageneric levels: no infrageric classification except some regional treatment has been proposed since Warburg and Reiche (1895). Warburg and Reiche (1895) recognized 14 sections in *Impatiens* based on phyllotaxy, the type of inflorescence, the number of flowers, and the length of the spur. Hooker (1904–1906) proposed some ‘groups’ for British Indian species also based on phyllotaxy, the type of inflorescence and the number and size of the flowers. Grey-Wilson (1980) considered the classification proposed by Warburg and Reiche (1895) to be artificial. Moreover Akiyama and Ohba (2000) have proved that the shape of the lower sepal (including the spur) has no good correlation with the basic chromosome number and structure of the inflorescence for the Himalayan species.

The chromosome numbers also show wide variation, n = 3 to 33 (Shimizu 1984, Zinov'eva-Stahevitch and Grant 1984, Akiyama, Wakabayashi and Ohba 1992). They suggested that the basic chromosome number is important for characterizing the Himalayan species. Akiyama and Ohba (2000) suggested that the relationship between the inflorescence type and the chromosome number and karyotype is interesting and that the inflorescence type might be an important character for recognizing infrageneric natural assemblages.

According to Jones and Smith (1966) the Himalayan region represents the center of origin of *Impatiens*. Grey-Wilson (1980) recognized two major groups based on the shape of the fruits: a northerly group with long-linear fruits and a southerly group with fusiform fruits. The two groups overlap significantly only in Southeast Asia (Burma, Indochina and southern China in the main). The Sino-Himalayan region is the most important area for resolving the origin and relationship of the species of *Impatiens*. Nevertheless, since Hooker (1904–1906) there have been no comprehensive systematic studies of the Sino-Himalayan species. Hara (1965, 1975), Grey-Wilson (1989a, 1989b), Akiyama (et al.) (1987, 1991, 1992, 1993, 1999) and other workers have been published critical studies for a only limited number of species and no phylogenetic study has been carried out on the Sino-Himalayan species of *Impatiens*. To consider significance of the Sino-Himalayan species of *Impatiens* we reveal the phylogenetic relationships of the species based on molecular phylogenetic analysis and discuss the correlation with the basic numbers of chromosomes and the type of inflorescences and lower sepals.

Materials and Methods

Origin of plant samples—For molecular phylogenetic analysis using DNA, we selected 26 accessions representing 25 species of *Impatiens* (Table 1). They were mainly collected in the Sino-Himalayan region (central and eastern Nepal and Yunnan) and most of them were also used for morphological and cytological studies (Akiyama, Ohba and Wakabayashi 1991, Akiyama, Wakabayashi and Ohba 1992, Akiyama and Ohba 2000). Voucher specimens, identified by S. Akiyama and H. Ohba are in the Herbarium of the University of Tokyo (TI).

Outgroup selection — Morton et al. (1996) indicated that the sister groups of Balsaminaceae were Marcgraviaceae and Tetrameristaceae based on molecular phylogenetic studies using *rbcL* sequences. However, Morton's report contained no data about ITS or *trnL-trnF* spacer sequences.

In the analysis of the relationships among species in the *Impatiens* we could not get materials of Marcgraviaceae as the outgroup. So as a preliminary study we chose *Tropaeolum majus* L. as the outgroup. Morphologically Balsaminaceae has been considered to be related to Tropaeolaceae in Geraniales because of the tenuinucellate ovule and nuclear endosperm (Tahktajan 1980). DNA was taken from fresh leaves of and a voucher specimen is deposited in TI (Table 1).

DNA extraction—Fresh leaves or leaves taken from specimens dried without heat were placed in plastic bags filled with silica gel and stored at –20 °C in a refrigerator. Total genomic DNA was isolated using a CTAB (hexadecyltrimethyl-ammonium bromide) procedure (Doyle and Doyle 1987).

PCR amplification and conditions for sequencing—In this study, three DNA regions were analyzed. The *rbcL* region and the spacer region between *trnL* (UAA) 3'ex on and *trnF* (GAA) (the *trnL/F* spacer) are regions of cpDNA, and the ITS1 region be-

Table 1. Taxa examined for cpDNA *rbcL*, and the spacer between *trnL* and *trnF* and nuclear rDNA ITS1 sequence variation in this study. Sequence data, except for ITS1, were deposited with Genbank. ITS1 sequence data were not analyzed phylogenetically. Symbols for taxon names were used in Figs. 2–7. Vouchers all at TI

Symbol	Taxon	Voucher	Source	Accession no.	
				rbcL	*trnL/F* spacer
AM	*Impatiens amplexicaulis* Edgew.	Miyamoto et al. 9485288	CENTRAL NEPAL: Gorkha Distr.	AB043532	AB043663
AR	*Impatiens arguta* Hook.f. & Thomson	Miyamoto et al. 9592060	EAST NEPAL: Solukhumbu Distr.	AB043509	AB043639
C1	*Impatines arguta* Hook.f. & Thomson	Wu et al. 1414	CHINA: Yunnan, Zhongdian Xian	AB043511	AB043640
H	*Impatines balfourii* Hook.f.	M. Nakao, 21-27 Sept. 1998	JAPAN: Hokkaido	AB043514	AB043653
C3	*Impatines barbata* Comber	Wu et al. 1413	CHINA: Yunnan, Zhongdian Xian	AB043530	AB043658
BI	*Impatines bicornuta* Wall.	Miyamoto et al. 9584016	CENTRAL NEPAL: Ramechhap Distr.	AB043510	AB043644
N1	*Impatines cymbifera* Hook.f.	Miyamoto et al. 9420007	CENTRAL NEPAL: Rasuwa Distr.	AB043517	AB043654
D	*Impatines discolor* DC.	Miyamoto et al. 9584026	EAST NEPAL: Solukhumbu Distr.	AB043533	AB043664
F	*Impatines falcifer* Hook.f.	Miyamoto et al. 9596504	EAST NEPAL: Solukhumbu Distr.	AB043513	AB043651
G1	*Impatines hawkeri* Bull.	H. Fujihashi 4	Cult. in Tokyo (origin: New Guinea)	AB043531	AB043643
K	*Impatines hoehnelii* T.C.E.Fr.	H. Ohba 990102	KENYA: Mt. Kenya	AB043515	AB043642
N	*Impatines noli-tangere* L.	H. Fujihashi 2	JAPAN: Kanagawa Pref.	AB043516	AB043659
C2	*Impatines nubigena* W.W.Sm.	Wu et al. 1001	CHINA: Yunnan, Zhongdian Xian	AB043529	AB043656
OC	*Impatines occultans* Hook.f.	Miyamoto et al. 9584111	EAST NEPAL: Solukhumbu Distr.	AB043518	AB043650
PR	*Impatines pradhanii* H.Hara	Miyamoto et al. 9485187	CENTRAL NEPAL: Gorkha Distr.	AB043519	AB043648
PU	*Impatines puberula* DC.	Miyamoto et al. 9584062	EAST NEPAL: Solukhumbu Distr.	AB043520	AB043662
RA	*Impatines racemosa* DC.	Miyamoto et al. 9596622	EAST NEPAL: Solukhumbu Distr.	AB043521	AB043645
RD	*Impatines radiata* Hook.f.	Miyamoto et al. 9584053	EAST NEPAL: Solukhumbu Distr.	AB043522	AB043649
SE	*Impatines serrata* Benth. ex Hook.f. & Thomson	Miyamoto et al. 9420180	CENTRAL NEPAL: Rasuwa Distr.	AB043523	AB043652
C4	*Impatines subecalcarata* Y.L.Chen	Wu et al. 1200	CHINA: Yunnan, Zhongdian Xian	AB043512	AB043657
SU	*Impatines sulcata* Wall.	Miyamoto et al. 9584319	EAST NEPAL: Solukhumbu Distr.	AB043524	AB043655
T	*Impatines textorii* Miq.	H. Fujihashi 1	JAPAN: Nagano Pref.	AB043525	AB043660
UN	*Impatines uniflora* Hayata	T. Kawasaki 7190	TAIWAN: Chiayi Hsien, Alishan	AB043526	AB043661
UT	*Impatines urticifolia* Wall.	Miyamoto et al. 9592558	EAST NEPAL: Solukhumbu Distr.	AB043527	AB043647
A1	*Impatines walleriana* Hook.f.	H. Fujihashi 3	Cult. in Tokyo (origin: Africa)	AB043508	AB043641
W	*Impatines wallichii* Hook.f.	Miyamoto et al. 9584060	EAST NEPAL: Solukhumbu Distr.	AB043528	AB043646
o-Tro	*Tropaeolum majus* L.	H. Fujihashi 5	Cult. in Tokyo (origin: South America)	AB043534	AB043665

tween 18S ribosomal DNA (rDNA) and 5.8S rDNA is a region of nuclear DNA. Double stranded (ds) DNAs of each region were amplified by the polymerase chain reaction method (PCR) using the primer sets containing the forward and the reverse that hybridize with the 5' end of each chain of dsDNAs in each region.

For the *rbcL* region, three primer sets were used; the front part of *rbcL*: 1-1 (5'-ATG TCA CCA CAA ACA GAG ACT AAA G C-3') and 1-2 (5'-CAT AAA CGG CTC TAC CGT AGT TTT TAG C-3') (Hasebe et al. 1994), the middle: OTNP1 (5'-TAT CCA TTG GAC CTT TTT GAA GAA GGT TC-3') and OT2PR (5'-GTA GTA GGT AAA CTA GAA GGA GAA AGA GA-3') (Endo, unpublished), the back: N2-1 (5'-TG A AAA CGT GAA TTC CCA ACC GTT TAT GCG-3') and NN3-2 (5'-GCA GCA GCT AGT TCC GGG CTC CA-3') (Hasebe et al. 1994). Each set covers more than a third length of the entire length. Similarly, the *trnL/F* spacer region required a primer set; B49873 (5'-GGT TCA AGT CCC TCT ATC CC-3') and A50272 (5'-ATT TGA ACT GGT GAC ACG AG-3') (Taberlet et al. 1991). The ITS1 region also required a primer set; ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3') (White et al. 1990).

The PCR protocol was the same for *rbcL*, the spacer, and ITS1. The reaction mixture contained 2–5 μL of purified genomic DNA (<1 μg), 1 μL of each primer (50 pmol each), 5 μL of 10×PCR buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$; Takara Shuzo, Shiga, Japan), 4 μL of dNTP mixture (2.5 mM of each dNTP), 0.25 μL of TaKaRa Taq™ (5 units/mL *Thermus aquaticus* DNA polymerase, 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 m M DTT, 0.5 % Tween®20, 0.5 % Nonidet P-40®, 50 % glycerol; Takara Shuzo, Shiga, Japan) was added up sterile distilled water to 50 μL of the total reaction volume. PCR conditions were: 1 min at 94 °C; 40 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C; 1 min at 72 °C; cooling and storing at 4 °C (GeneAmp® PCR System 2400; PE Biosystems, Applied Biosystems Division, Foster City, CA, USA). The products of PCR were visualized in 1 % agarose gels stained with ethidium bromide (EtBr), excised from agarose gel and purified using the GENECLEAN II Kit (BIO Sequencing protocol).

Purified PCR products were sequenced using a dye-terminator cycle sequencing method (BigDye Terminator Cycle Sequencing FS Ready Reaction Kit; PE Biosystems, Applied Biosystems Division, Foster City, CA, USA) with AmpliTaq DNA polymerase, FS, according to the protocol. The *rbcL* region was divided into three parts which were amplified separately. The other regions were also analyzed using the same primers as PCR. The total 10 μL of each reagent contained 5 pmol of the primer, about 300 ng of the PCR products, 4 μL pre-mix from the kit and 1 μL of sterile distilled water. After free dye-terminator was removed using DyeEx™ Spin kit (QIAGEN, Valencia, CA, USA), cycle sequencing products were analyzed on an ABI 310 Genetic Analyzer (PE Biosystems, Applied Biosystems Division, Foster City, CA, USA).

Sequence analysis—All sequences were confirmed by comparison of their forward and reverse sequences. Because the published *rbcL* sequences of taxa belonging to *Impatiens* are very few, sequence boundaries of the *rbcL* region of all taxa were determined by comparison of sequence data of all taxa in this study and *Impatiens capensis* (Chase et al. 1993). There are no reports for the ITS1 and the *trnL/F* spacer region of *Impatiens*, so the sequence boundaries were also determined by comparison of all taxa in this study.

All sequences of the three regions were determined at first with Factura ver. 2.2 and Sequence Navigator ver. 1.0 software (PE Biosystems, Applied Biosystems Division, Foster City, CA, USA). These sequences were then aligned with CLUSTAL X software (Thompson et al. 1994). Because of heavy sequential mutations or modification, the ITS1 sequences were aligned with DIALIGN 2 software (Morgenstern 1999, Morgenstern et al. 1996, 1998, http://genomatix.gsf.de/cgi-bin/dialign/). Finally, these alignments were adjusted visually on Sequence Navigator ver. 1.0 (PE Biosystems, Applied Biosystems Division, Foster City, CA, USA).

The homologies of each regions were calculated with DnaSP ver. 3.0 (Rozas and Rozas 1999).

Phylogenetic analysis—Sequence data of 26 ingroup samples of 25 species and one outgroup samples of one species were analyzed using PAUP* ver. 4.0beta (Swofford 1998). All characters were unordered and equally weighted. Gaps were coded as missing data and multistate taxa were interpreted as uncertain. Phylogenetic trees were generated by two methods using PAUP software. One was the most parsimonious (MP) method using the heuristic search method with tree bisection-reconnection (TBR) swapping algorithm. The other was the neighbor joining (NJ) method using Kimura-2-parameter pairwise distance matrix (Kimura 1980).

To estimate the phylogenetic trees drawn by PAUP, bootstrap analyses were performed. For the MP method, bootstrap values were calculated and set to full heuristic search with TBR swapping algorithm and simple addition sequence of 100 replicates. For NJ method, those were calculated by 1000 replicates. Additionally, fit measures of phylogenetic trees to sequence data, the consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated.

Phylogenetic trees generated by the NJ method were only used to check topological relationships among sample taxa in MP trees. The phylogenetic trends and morphological evolution is discussed mainly on the basis of the molecular phylogenetic tree resulting from the MP method.

Results

Sequences—Data matrixes of three regions were aligned completely. Their length resulted in the 1291 bp of *rbcL* homologous region (Genebank accession no. AB043508–AB043534), the 344–412 bp of *trnL/F* spacer homologous region (Genebank accession no. AB043639–AB043665), and the 277–297 bp of ITS1 region. The data matrix of the ITS1 region had so many sites with high sequential diversities that phylogenetic analysis could not be determined for it (Fig. 1).

Phylogenetic relationships within *Impatiens*—The molecular phylogenetic analysis within *Impatiens* was carried out using *rbcL*, *trnL/F*, ITS1, and preliminary ITS2 and *matK* regions. ITS2 and *matK* regions were not analyzed in all samples (data not shown). All analyzed ITS1 sequences had high sequential diversities (Fig. 1), so *rbcL* and *trnL/F* regions in all samples were used to infer phylogenetic relationships within *Impatiens*.

Parsimony analysis of the *rbcL* sequences yielded a single most parsimonious (MP) tree of *Impatiens* (L = 249 steps, CI = 0.651, RI = 0.620 and RC = 0.403) (Fig. 2). Branches of the tree were drawn by 50 % majority rules with 100 replicate bootstrap analyses. The analysis of the *trnL/F* spacer sequences also yielded a single most parsimonious tree of *Impatiens* (L = 229 steps, CI = 0.830, RI = 0.832 and RC = 0.691) with bootstrap 50 % majority rules (Fig. 4). In the *trnL/F* MP tree, *Impatiens* was divided into two clades (clades A and B). Bootstrap val-

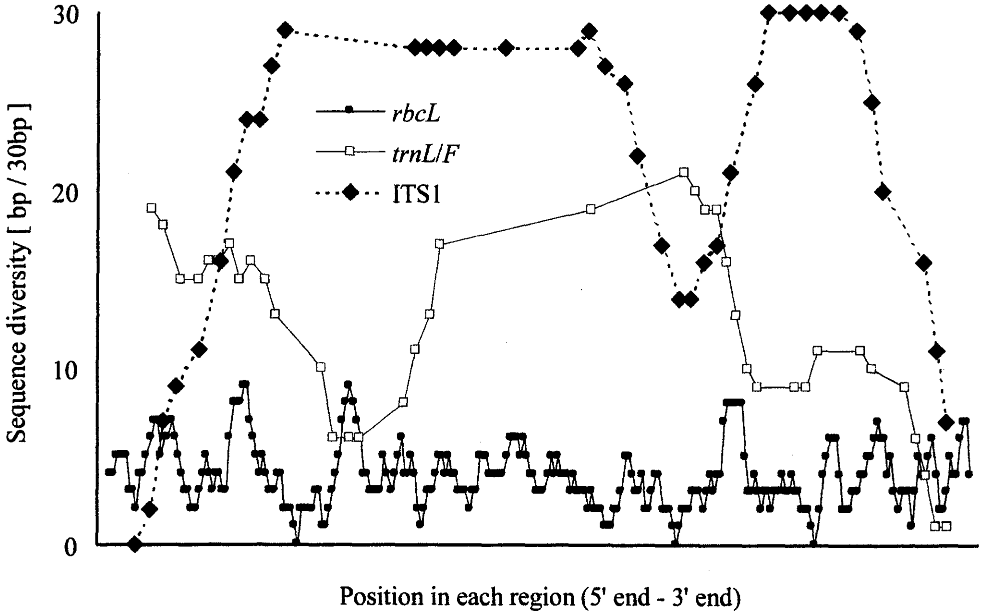


Fig. 1. Sequence diversity of *rbcL*, *trnL/F* and ITS1 examined from 5'end to 3'end from 26 samples of ingroup taxa and 1 sample of outgroup taxon in each 30 bp with 5 bp steps.

ues of these two clades were 85 % (for A) and 76 % (for B) (Fig. 4). Clade A was supported in the *rbcL* MP tree (bootstrap value: 75 %) while clade B collapsed (Fig. 2). Trees by NJ method were drawn to estimate tree forms by MP method. Both NJ trees (*rbcL* and *trnL/F*) (Figs. 3, 5) supported two clades, A and B, and these bootstrap values of 1000 replicate were A: 81 % and B: 52 % in the NJ tree of *rbcL* (Fig. 3), and A: 50 % and B: 77 % in of *trnL/F* (Fig. 5).

Sequence data matrix of *trnL/F* contained six conspicuous indels owned by two or more species. The modified data matrix added these indels as new characters weighted equivalently were treated in the same way as the MP tree of unmodified data. The result showed almost equal topology to unmodified *trnL/F* data (data not shown). It was suggested that the taxa which owned the six indels have genetically close relationship.

Phylogenetic relationships within *Impatiens* in this study were determined as a single MP tree (L = 445 steps, CI = 0.791, RI = 0.799 and RC = 0.632) derived from the combined data matrix of *rbcL* and *trnL/F* (Fig. 6). The MP tree contained same two clades as the MP tree of the single data matrix of *trnL/F* (Fig. 4). Bootstrap values were 90 % (clade A) and 82 % (clade B). The NJ tree of this combined data also supported these two clades (bootstrap value of A: 91 % ; B: 89 %) (Fig. 7).

Clade A comprised three monophyletic groups (A1, A2, A3), *Impatiens balfourii* and *I. cymbifera*. Group A1 consists of *I. radiata*, *I. pradhanii*, *I. occultans*, *I. wallichii*, *I. urticifolia*, *I. racemosa* and *I. bicornuta* (bootstrap value: 98 %). Group A2 consists of *I. amplexicaulis* and *I. sulcata* (bootstrap value: 89 %). Group A3 consists of *I. falcifer* and *I. serrata* (bootstrap value: 99 %).

Samples within clade B were divided into four monophyletic groups (B1, B2, B3, B4). Group B1 was the most major group in clade

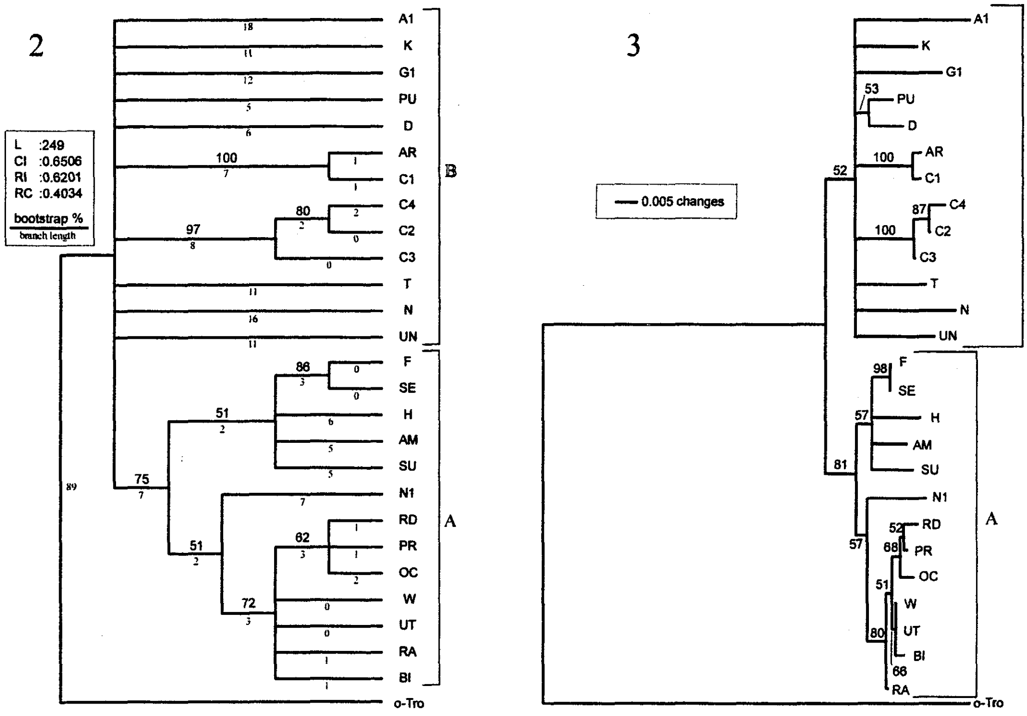


Fig. 2–3. Relationships of Sino-Himalayan *Impatiens* based on *rbcL* sequence data matrix. 2. The single minimum length most parsimonious (MP) tree. Bootstrap values (100 replicates) are indicated above the branches and branch length; number of nucleotide substitutions is indicated below them. L, CI, RI and RC indicate full length of tree, consistency index, retention index and rescaled consistency index. 3. Tree obtained from neighbor-joining (NJ) method. Number near branches is percentage of bootstrap values (1000 replicates). Scale of branches indicates number of nucleotide substitutions per site calculated by Kimura's two parameter method (Kimura 1980).

B, consisting of *I. subecalcarata*, *I. nubigena*, *I. barbata*, *I. textorii*, *I. noli-tangere* and *I. uniflora* (bootstrap value: 64 %). In clade B, group B2 contains only one species, *I. arguta*, from two populations from Nepal and China (bootstrap value: 100 %). Group B3 consists of *I. puberula* and *I. discolor* (bootstrap value: 100 %). The remaining species, *I. walleriana*, *I. hoehnelii* and *I. hawkeri*, belong to group B4 (bootstrap value: 100 %). Among these groups, monophyly was implied in B2, B3 and B4, but statistically, it was supported weakly (bootstrap value: 48 %). Groups B3 and B4 were closely related to each other (bootstrap value: 75 %).

Discussion

Sequential diversity in *Impatiens*—The ITS1 sequences of *Impatiens* are so complex that we could not decide where the homologous area in the ITS1 region is (Fig. 1). The family Balsaminaceae probably required a long time to acquire its wide diversity since the ancestral species appeared among angiosperms. In general ITS sequences are useful for clarifying inter-species to inter-generic phylogenetic reconstruction, (Soltis et al. 1998) but those of *Impatiens* are not useful. Moreover, in general the cpDNA *rbcL* se-

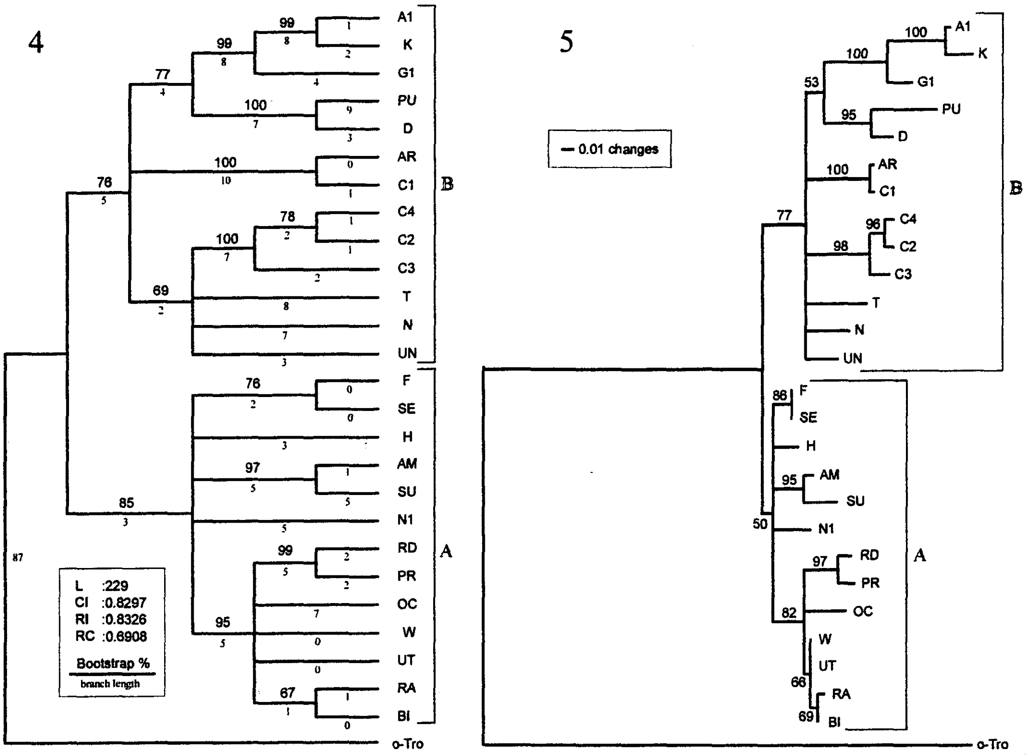


Fig. 4–5. Relationships of Sino-Himalayan *Impatiens* based on the *trnL/F* spacer sequence data matrix. 4. The single minimum length most parsimonious (MP) tree. Bootstrap values (100 replicates) are indicated above branches; branch length (number of nucleotide substitutions) is indicated below them. L, CI, RI and RC indicate full length of tree, consistency index, retention index and rescaled consistency index. 5. Tree obtained from neighbor-joining (NJ) method. Number near branches is percentage of bootstrap values (1000 replicates). Scale of branches indicates number of nucleotide substitutions per site calculated by Kimura's two parameter method (Kimura 1980).

quences are well used to refer to higher taxonomical rank than genus (Soltis et al. 1998) but those of *Impatiens* have many sequential variations (mutations or indels) enough to analyze genetical relationships among species.

Phylogenetic relationships and chromosome numbers within *Impatiens*—The molecular phylogenetic analysis of regions of the *rbcL* gene and the spacer *trnL/F* divide *Impatiens* into two clades and seven groups (Fig. 6). In this study we discuss the cytotaxonomic data about chromosome number, distribution and the seven groups derived from this molecular phylogenetic tree of *rbcL* and *trnL/F* sequences (Fig. 8).

Monophyly of clade A is supported by all analyses with high values of statistical potential. This clade contains species from the Himalayan region. In this clade three small monophyletic groups can be recognized on the basis of chromosome number: species belonging to group A1 have 2n = 18 chromosomes (x = 9), species of group A2 have 2n = 18 (x = 9) chromosomes, and species of group A3 have 2n = 14 (x = 7) chromosomes. Clade B and clade A are monophyletic. In clade B, group B4 is in

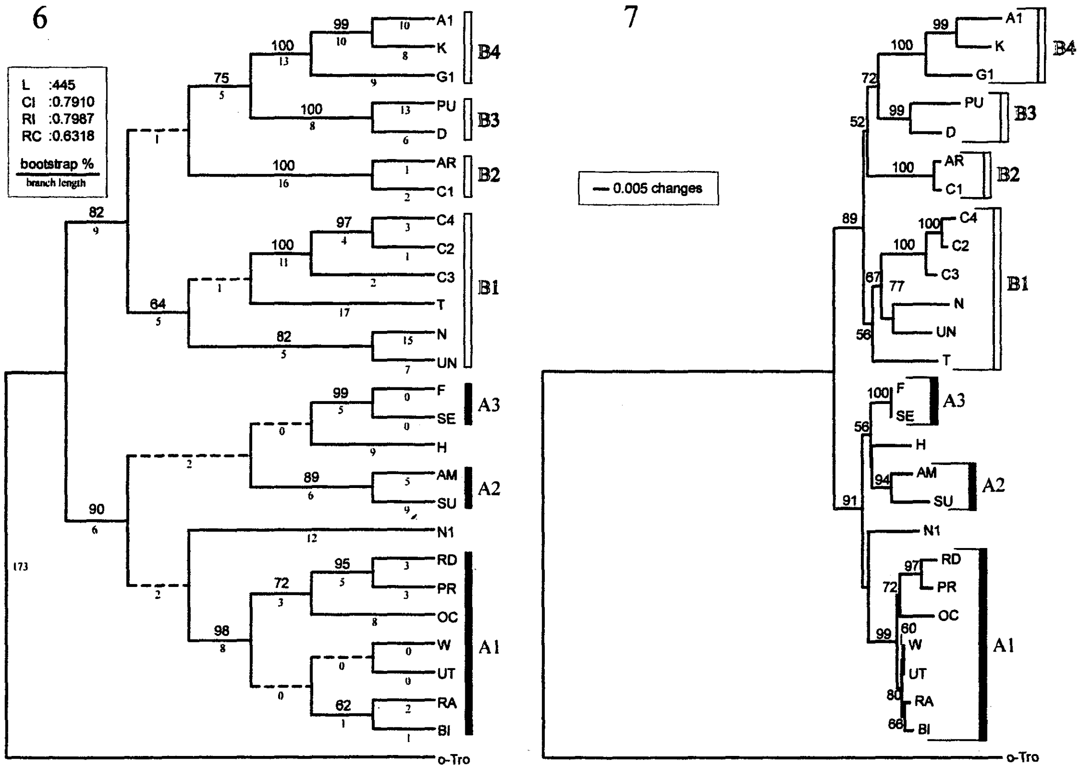
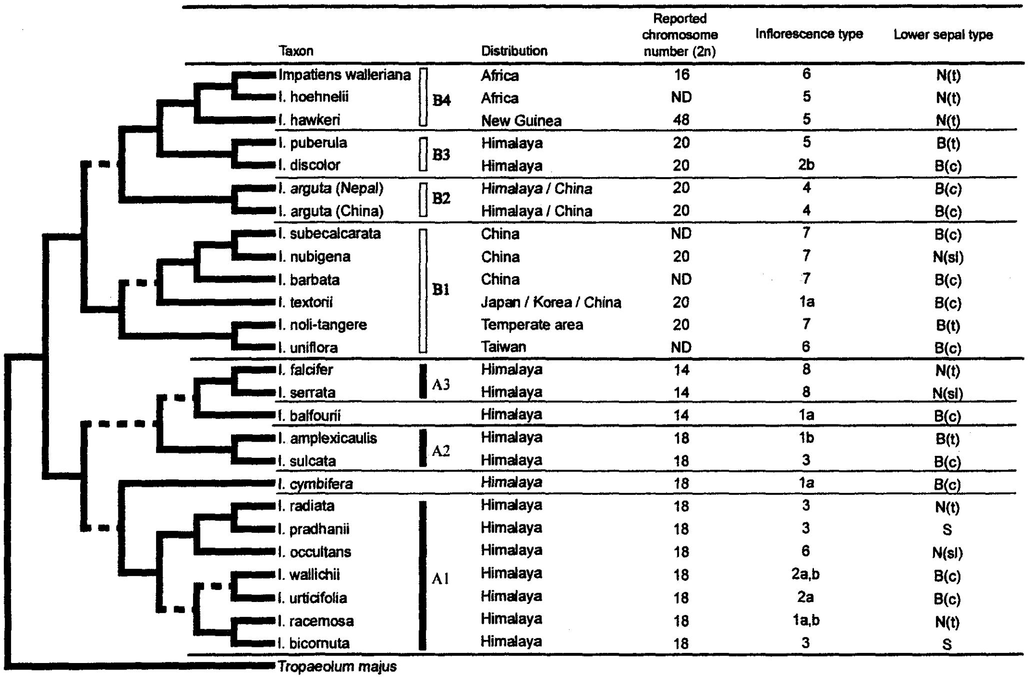


Fig. 6–7. Relationships of Sino-Himalayan *Impatiens* based on combined sequence data matrix of *rbcL* and *trnL/F* spacer sequences. 6. Single minimum length most parsimonious (MP) tree. Bootstrap values (100 replicates) are indicated above branches; branch length (number of nucleotide substitutions) is indicated below them. Branches with less than 50 % bootstrap value are represented by dotted lines. L, CI, RI and RC indicate full length of tree, consistency index, retention index and rescaled consistency index. 7. Tree obtained from neighbor-joining (NJ) method. Number near branches is percentage of bootstrap value (1000 replicates). Scale of branches indicate number of nucleotide substitutions per site calculated by Kimura's two parameter method (Kimura 1980).

Africa and New Guinea and includes species with 2n = 16 (x = 8) (*I. walleriana*) and 2n = 48 (x = 8) (*I. hawkeri*). Species of group B1 are in southern China and Japan; species of group B2 are in southern China and the Himalaya, and species of group B3 in the Himalaya. Groups B1, B2, and B3 have 2n = 20 (x = 10) chromosomes.

The tree and chromosome numbers (Fig. 8) indicate that all species of *Impatiens* originated from an ancestral species that divided into two clades: the type A clade with x = 7 or 9 and the type B clade with x = 8 or 10. We call clades A and B the Himalayan clade and Chinese clade, respectively. It is presumed that the African and New Guinean species differentiated from the Chinese clade. If so, species with x = 8, which is the major chromosome number in African *Impatiens*, are derived from species with x = 10.

Some species of B clade, B2, extend their range to Himalaya via China (Yunnan and Sichuan), those of B3 are endemic to Himalaya. This means that the present Himalayan flora of *Impatiens* comprises two



Taxon		Distribution	Reported chromosome number (2n)	Inflorescence type	Lower sepal type
Impatiens walleriana	B4	Africa	16	6	N(t)
I. hoehnelii		Africa	ND	5	N(t)
I. hawkeri		New Guinea	48	5	N(t)
I. puberula	B3	Himalaya	20	5	B(t)
I. discolor		Himalaya	20	2b	B(c)
I. arguta (Nepal)	B2	Himalaya / China	20	4	B(c)
I. arguta (China)		Himalaya / China	20	4	B(c)
I. subecalcarata	B1	China	ND	7	B(c)
I. nubigena		China	20	7	N(sl)
I. barbata		China	ND	7	B(c)
I. textorii		Japan / Korea / China	20	1a	B(c)
I. noli-tangere		Temperate area	20	7	B(t)
I. uniflora		Taiwan	ND	6	B(c)
I. falcifer	A3	Himalaya	14	8	N(t)
I. serrata		Himalaya	14	8	N(sl)
I. balfourii		Himalaya	14	1a	B(c)
I. amplexicaulis	A2	Himalaya	18	1b	B(t)
I. sulcata		Himalaya	18	3	B(c)
I. cymbifera		Himalaya	18	1a	B(c)
I. radiata	A1	Himalaya	18	3	N(t)
I. pradhanii		Himalaya	18	3	S
I. occultans		Himalaya	18	6	N(sl)
I. wallichii		Himalaya	18	2a,b	B(c)
I. urticifolia		Himalaya	18	2a	B(c)
I. racemosa		Himalaya	18	1a,b	N(t)
I. bicornuta		Himalaya	18	3	S
Tropaeolum majus					

Fig. 8. Relationships of taxonomic and cytotaxonomic characters from previous studies and MP tree based on combined sequence data matrix of *rbcL* and *trnL/F* spacer (Fig. 6). Inflorescence types and lower sepal types follow Akiyama and Ohba (2000). Chromosome counts for *Impatiens nubigena* and *I. amplexicaulis* are unpublished.

fundamentally different ancestors with different chromosomal basic numbers. No Himalayan *Impatiens* of A clade were found in China. The species which range both Himalaya and China are all of B clade.

Morphological evolution of the inflorescence and floral organs—The morphological features of *Impatiens* stand out in the flower and the inflorescence. The distinctive zygomorphic entomophilous flowers in which lower sepal with a nectariferous spur shows coevolutionary relationships to pollinators (Grey-Wilson 1980). The length of the spur and the type of the inflorescence were regarded as key characters for recognizing infrageneric taxa (Warburg and Reiche 1895, Hooker 1904–06). The shape of the inflorescence, however, is complicated, Akiyama and Ohba (2000) recognized nine types in the Himalayan species of *Impatiens*.

Adapting Akiyama and Ohba's nine inflorescence types (Akiyama and Ohba 2000) for the molecular tree (Fig. 8), group A3 has type 8 and four species of B1 have type 7. Type 7 and type 8 are unique in the Himalayan *Impatiens*. Many species of clade A have type 1, 2, 3, or 8 inflorescences while many of clade B have type 4, 5, 6, or 7. Type 1, 2, 3 are raceme or modified raceme and considered to be ancestral features. In contrast type 8 is unique one and restricted only in group A3. It is considered that type 8 is advanced stage. Type 4 and 5 have very short peduncle and rachis and are considered to be derived ones (Grey-Wilson 1980), and are restricted only in clade B.

There is a low correlation between the

molecular phylogenetic tree and lower sepal morphology (Fig. 8). The morphology of the lower sepal is probably diversified at the species level with relationships to pollinator as suggested by Akiyama and Ohba (2000).

From the present study it is presumed that the present Sino-Himalaya flora of *Impatiens* comprises two fundamentally different ancestors, one is the Himalayan clade and the other the Chinese clade, and that from one of them, the Chinese clade, the east Asian species and the African and New Guinean species differentiated. To establish the infrageneric classification with the molecular phylogenetic analysis (*rbcL* and *trnL/F* sequences) the characters of the chromosome numbers and the gross morphology are important.

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藤橋弘朋[a]，秋山　忍[b]，大場秀章[a]：**中国ーヒマラヤ産ツリフネソウ属（ツリフネソウ科）の起源と系統関係**

中国ーヒマラヤ産ツリフネソウ属の系統関係について *rbcL* と *trnL-trnF* 間の遺伝子領域の塩基配列を用いて解析を行った．その結果，2つの単系統群（クレードAとクレードB）が認められた．クレードAはヒマラヤ産の種のみからなるのにたいして，クレードBは中国その他いろいろな地域産の種からなることが判った．得られた系統樹は染色体基本数との相関が高く，クレードA（ヒマラヤクレードと呼ぶ）の基本染色体数は $x = 7$, 9であるのに対して，クレードB（中国クレードと呼ぶ）は基本染色体数 $x = 8$，10である．この中国クレードには日本産を含めた東アジア産の種および北半球温帯産の種からなるサブクレード，中国・ヒマラヤ産の種からなるサブクレード，ヒマラヤ産の種からなるサブクレード，アフリカ産とニューギニア産の種からなるサブクレードがみとめられる。他の形態形質では，花序形態がこの系統樹と高い相関を示している．これらのことからツリフネソウ属では，染色体基本数と花序形態が系統進化を反映しているといえる．

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